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RESEARCH ARTICLE

Human Achilles tendon plasticity in response to cyclic strain: effect of rate and duration

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ABSTRACT

High strain magnitude and low strain frequency are important stimuli for tendon adaptation. Increasing the rate and duration of the applied strain may enhance the adaptive responses. Therefore, our purpose was to investigate the effect of strain rate and duration on Achilles tendon adaptation. The study included two experimental groups (*N*=14 and *N*=12) and a control group (*N*=13). The participants of the experimental groups exercised according to a reference protocol (14 weeks, four times a week), featuring a high strain magnitude (~6.5%) and a low strain frequency (0.17 Hz, 3 s loading/3 s relaxation) on one leg and with either a higher strain rate (one-legged jumps) or a longer strain duration (12 s loading) on the other leg. The strain magnitude and loading volume were similar in all protocols. Before and after the interventions, the tendon stiffness, Young's modulus and cross-sectional area were examined using magnetic resonance imaging, ultrasound and dynamometry. The reference and long strain duration protocols induced significantly increased (P<0.05) tendon stiffness (57% and 25%), cross-sectional area (4.2% and 5.3%) and Young's modulus (51% and 17%). The increases in tendon stiffness and Young's modulus were higher in the reference protocol. Although region-specific tendon hypertrophy was also detected after the high strain rate training, there was only a tendency of increased stiffness (P=0.08) and cross-sectional area (P=0.09). The control group did not show any changes (P=0.86). The results provide evidence that a high strain magnitude, an appropriate strain duration and repetitive loading are essential components for an efficient adaptive stimulus for tendons.

KEY WORDS: Exercise, Load, MRI, Tendon adaptation, Tendon training, Tendon hypertrophy

INTRODUCTION

Tendinous tissue is highly sensitive to mechanical loading. The external strain of the tendon following contractions of the attached muscle is transmitted through the extracellular matrix on the cytoskeleton of the mechanosensitive tendon cells via membrane proteins (e.g. integrins, G-proteins, receptors and protein kinases) (Wang, 2006). The deformation of tendon cells initiates the expression of genes responsible for catabolic and/or anabolic cellular and molecular responses (e.g. collagen synthesis), which affect the mechanical (stiffness), morphological (cross-sectional area) and material (Young's modulus) tendon properties (Galloway et al., 2013; Heinemeier and Kjaer, 2011; Kjaer, 2004; Lavagnino and Arnoczky, 2005; Wang, 2006). From a mechanobiological point of view, four factors of the applied strain may affect the adaptive response of tendons: magnitude, frequency, rate and duration (Arnoczky et al., 2002; Lavagnino et al., 2008; Yamamoto et al., 2005; Yamamoto et al., 2003; Yang et al., 2004). Recent experiments on the human Achilles tendon (AT) *in vivo* by our group showed that a high strain magnitude (4.5–5.0%) is required to trigger adaptive effects on the tendon mechanical, morphological and material properties (Arampatzis et al., 2010; Arampatzis et al., 2007). Furthermore, we found that applying the same high strain magnitude with a low strain frequency (i.e. 0.17 Hz, 3 s loading/3 s relaxation versus 0.5 Hz, 1 s loading/1 s relaxation) leads to superior adaptive responses of the tendon properties (Arampatzis et al., 2010). However, the possibility of a superimposed effect of the applied tendon strain rate and tendon strain duration in relation to a high strain magnitude and a low strain frequency on the plasticity of the mechanical, morphological and material tendon properties of humans *in vivo* has not been investigated yet. A profound understanding of tendon plasticity is essential, particularly with regard to tendon adaptation and tendon healing. Such information could allow for improvements in human locomotor performance, as well as tendon injury prevention and rehabilitation.

The cellular adaptive responses of tendons are dependent on the transmission of the external tendon strain via the extracellular matrix to the mechanosensitive tendon cells. The strain at the cellular level is much lower compared with the external tendon strain (Arnoczky et al., 2002; Screen et al., 2005). Two modes were suggested for the transmission of the external strain to the cellular level: cell deformation and fluid flow-induced shear stress (Lavagnino et al., 2003; Lavagnino et al., 2008). With increasing strain, a loss of collagen crimp and an increase in fiber recruitment was observed (Hansen et al., 2002; Schatzmann et al., 1998) and likely results in an increased number of cells being deformed (Arnoczky et al., 2002). The inhibition of catabolic cell responses (collagenase mRNA) seems to be directly associated with progressive increases of strain magnitude (Arnoczky et al., 2004; Lavagnino et al., 2008; Lavagnino et al., 2003). Furthermore, the extracellular matrix features viscous behavior due to the content of collagen and water, and interactions between collagenous and non-collagenous properties (Ciarletta and Ben Amar, 2009; Wang, 2006), and, thus, a time-dependent transmission of the external tendon strain to the cytoskeleton. Therefore, we can argue that longer durations of tendon strain may more effectively transmit external strain on the cells and likely result in a greater cell deformation, thus providing a superior adaptive stimulus compared with shorter ones.

In addition to cell deformation, fluid flow-induced shear stress (hydrostatic stress) was suggested to be a stimulus that affects tendon adaptation (Archambault et al., 2002; Giori et al., 1993; Lavagnino et al., 2008; Lavagnino et al., 2003). *In vitro* experiments demonstrated the existence of interstitial fluid flow within the tendinous tissue following cyclic loading (Hannafin and Arnoczky, 1994; Helmer et al., 2006; Lanir et al., 1988). Load-related fluid

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List of symbols and abbreviations

AT	Achilles tendon
CSA	cross-sectional area
d	effect size
EMG	electromyography
MRI	magnetic resonance imaging
MTJ	m. gastrocnemius medialis myo-tendinous junction
MVC	maximal voluntary isometric plantar flexion force

flow was shown to induce a shear stress on the cell membrane, which stimulated the tendon cells to alter their gene expression (Archambault et al., 2002). Moreover, the applied tendon strain rate may determine the fluid flow-induced shear stress (Haut and Haut, 1997; Lavagnino et al., 2008). A recent study (Lavagnino et al., 2008) reported that an increase in strain rate from 2% min^{-1} to 20% min^{-1} using the same strain magnitude decreased the interstitial collagenase mRNA expression in rat tail tendon significantly (relative quantification of MMP-13: 1401 versus 33, respectively), indicating that strain rate-mediated fluid shear stress alone is efficient to inhibit catabolic gene expression. Therefore, increased strain rate may enhance the adaptive response of human tendons *in vivo*.

From a mechanobiological point of view, we can argue that tendon adaptation is a result of the close interaction of the mechanical environment and the biology of the tendinous tissue. With regard to this interaction, it is of great importance to understand the mechanical conditions (e.g. strain rate-mediated fluid shear stress and time-dependent cell deformation) that may influence the tendon adaptive responses *in vivo*, not only in applied but also in basic research. Hence, our previous (Arampatzis et al., 2010; Arampatzis et al., 2007) and present experiments concentrated on the identification of the most appropriate mechanics for biological responses (i.e. tendon adaptation). The purpose of the present study was to investigate whether increasing the strain rate or the strain duration within a high-magnitude loading regimen would provide a superimposed stimulus for the adaptation of the mechanical, morphological and material properties of the human AT *in vivo*. The strain rate and duration were modified with respect to a reference exercise protocol (i.e. high tendon strain magnitude and low strain frequency), which induced the most superior adaptive effects in our earlier studies (Arampatzis et al., 2010; Arampatzis et al., 2007). Because isometric muscle contractions, as in our previous studies (Arampatzis et al., 2007; Arampatzis et al., 2010), did not allow an adequate increase in the strain rate, the higher strain rate was achieved by means of impact loading (i.e. jumping). Based on the expected time dependency of the transfer from the external strain to the cellular level, we hypothesized that a longer tendon strain duration compared with the reference protocol would facilitate the adaptation of the mechanical, morphological and material tendon properties. Further, we hypothesized that a higher strain rate compared with the reference protocol may provide an additional adaptive stimulus and enhance the adaptive responses of the tendon, most likely through an associated increase of hydrostatic pressure and fluid flow-induced cell shear stress as suggested from *in vitro* studies.

RESULTS

Intervention 1: effect of strain rate

There was a significant effect of exercise intervention 1 on AT stiffness ($P<0.001$) as well as an interaction of the factors intervention and protocol ($P=0.024$). Stiffness increased significantly after the 14 weeks of exercise with the reference protocol ($P=0.009$, Fig. 1) while only a tendency towards higher values was found in the leg that was exercised using the high strain rate protocol ($P=0.081$, Fig. 1). The effect size of the AT stiffness increase in the reference and high strain rate protocol was $d=1.08$ and $d=0.69$, respectively. The stiffness values of the control group remained unchanged ($P=0.856$, Fig. 1). Regarding the 10% intervals of AT cross-sectional area (CSA) along AT length, we found a significant increase in the proximal part from 30% to 100% tendon length (30–40%: $P=0.008$, 40–50%: $P=0.031$, 50–60%: $P=0.003$, 60–70%: $P=0.005$, 70–80%: $P=0.002$, 80–90%: $P=0.002$, 90–100%: $P=0.019$) in the leg that was exercised by means of the reference protocol (Fig. 2). In the leg trained by the high strain rate protocol, we also found a significant region-specific hypertrophy of the AT, but just in the 20–30% ($P=0.005$), 30–40% ($P=0.013$), 60–70% ($P=0.033$) and 80–90% ($P=0.035$) intervals (Fig. 2). Furthermore, there was a significant effect of the intervention on the average CSA of the AT ($P<0.001$). The average CSA increased significantly after the completion of training using the reference protocol ($P<0.001$) and tended to increase ($P=0.089$) following training using the high strain rate protocol (Table 1). Further, the Young's modulus of the AT increased significantly in the reference protocol ($P=0.009$, Table 1) but not in the leg that was exercised using the high strain rate protocol ($P=0.194$, Table 1). The length of the free AT did not change following the intervention with either protocol (all $P>0.29$, Table 1). The effect sizes of the investigated parameters with regard to the respective protocols are presented in Table 1. The body mass of the participants of intervention group 1 and the control group did not change during the 14 weeks of training (intervention: 82.2±13 kg before training, 81.9±13.5 kg after training; control: 78.6±10.7 kg before, 78.3±10.4 kg after).

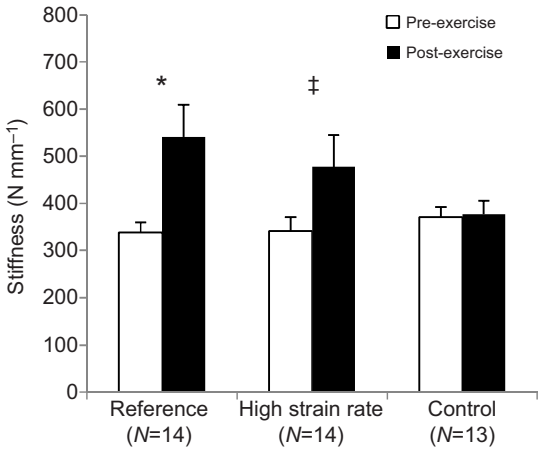

Fig. 1. Mean stiffness values and s.e.m. (error bars) of the Achilles tendon (AT) before (pre-exercise) and after (post-exercise) intervention 1, featuring the reference and high strain rate protocol as well as the control group. There was a statistically significant interaction of intervention and protocol ($P=0.024$). The *post hoc* comparisons show a significant increase in stiffness only in the reference protocol. *Statistically significant difference between the pre- and post-exercise values ($P=0.009$). ‡Tendency towards a difference between the pre- and post-exercise values ($P=0.081$).

Intervention 2: effect of strain duration

The exercise intervention 2 had a significant effect on the stiffness of the AT ($P<0.001$). Further, there was an interaction between the factors intervention and protocol ($P=0.002$) and the *post hoc* comparisons showed that the AT stiffness increased significantly ($P<0.001$) following both training protocols [i.e. reference

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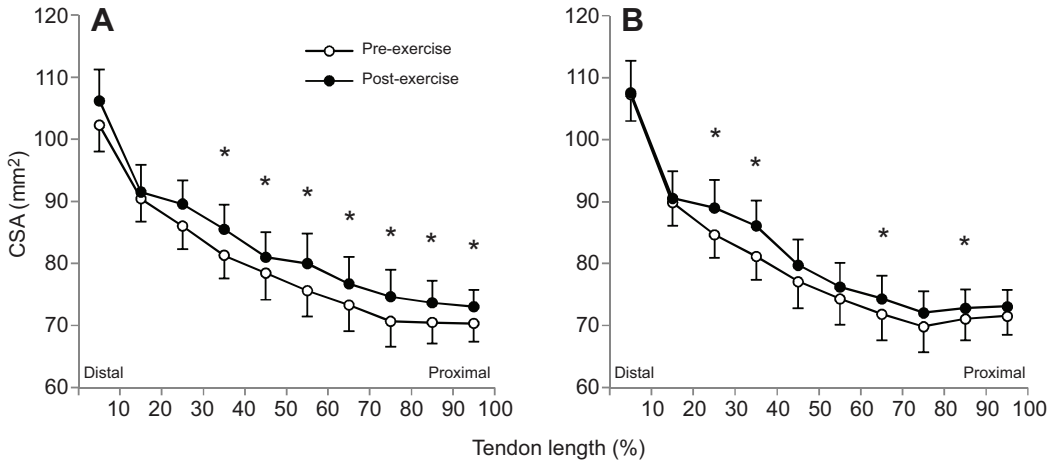

Fig. 2. Mean cross-sectional area (CSA) and s.e.m. (error bars) of the AT in 10% intervals of the tendon length before (pre-exercise) and after (post-exercise) intervention 1, featuring the reference and high strain rate protocol. (A) Reference protocol (*N*=14); (B) high strain rate protocol (*N*=14). *Statistically significant difference between the pre- and post-exercise values (*P*<0.05).

(*P*<0.001) and long strain duration (*P*=0.008)] but remained unchanged (*P*=0.856) in the control group (Fig. 3). The increase in AT stiffness was higher following the reference protocol than with the long strain duration protocol (*P*=0.025, Fig. 3). The effect size of the stiffness increase following the reference and long strain duration protocol was *d*=1.51 and *d*=0.68, respectively. The 10% interval analysis of the CSA along the tendon length showed a significant increase in the proximal part from 30% to 100% following training with both the reference (30–40%: *P*=0.003, 40–50%: *P*<0.001, 50–60%: *P*<0.001, 60–70%: *P*=0.002, 70–80%: *P*=0.004, 80–90%: *P*=0.007, 90–100%: *P*=0.007, Fig. 4) and long strain duration protocol (30–40%: *P*<0.001, 40–50%: *P*=0.006, 50–60%: *P*<0.001, 60–70%: *P*=0.002, 70–80%: *P*=0.006, 80–90%: *P*=0.007, 90–100%: *P*=0.015, Fig. 4). Furthermore, the average CSA increased significantly following both protocols (*P*<0.001, Table 2). There was a significant intervention effect on the Young's modulus of the AT (*P*<0.001). The values increased significantly following the reference (*P*<0.001) and long strain duration protocol training (*P*=0.047, Table 2). However, an interaction effect (intervention × protocol) indicated that the increase of the Young's modulus was more pronounced following the reference protocol (*P*=0.021, Table 2). The length of the free AT did not change following the intervention with either protocol (all *P*>0.19, Table 2). The effect sizes of the investigated parameters with regard to the respective protocol of intervention 2 are presented in Table 2. The body mass of the participants of intervention group 2 remained constant during the 14 weeks of training (74.8±7.3 kg before training, 75±7.2 kg after training).

DISCUSSION

The present study investigated the potential of a superimposed effect of strain rate and strain duration on the AT adaptation of healthy young male adults and completes our earlier experiments that focused on the effects of strain magnitude and strain frequency (Arampatzis et al., 2010; Arampatzis et al., 2007). Two 14 week interventions were conducted featuring a controlled modification of the strain rate and strain duration of the AT, respectively. The participants exercised according to a reference protocol, similar to the one that induced the most superior adaptive effects in our earlier experiments, on one leg, and either a comparatively higher strain rate (intervention 1) or longer strain duration (intervention 2) on the other leg. After completing the training using the reference and the long strain duration protocols, the subjects showed a clear increase of AT stiffness, average CSA and Young's modulus. However, the increase of AT stiffness and Young's modulus was more pronounced following the reference protocol compared with the long strain duration protocol. Although a region-specific hypertrophy of the tendon was also detected following the high strain rate protocol, average CSA and AT stiffness showed only a tendency towards higher values and the Young's modulus did not change at all. Based on these findings we have to reject both hypotheses.

In our initial hypotheses, we postulated that the increased hydrostatic pressure and fluid flow-induced shear stress within the tendinous tissue associated with a higher tendon strain rate (i.e. induced by one-legged jumps) (Giori et al., 1993; Haut and Haut, 1997; Helmer et al., 2006) would be an additional stimulus for tendon adaptation (Archambault et al., 2002; Giori et al., 1993; Lavagnino et al., 2008). However, the adaptive responses of the AT properties after the high strain rate protocol were lower compared with those following the reference protocol. Although the biological mechanism(s) for the reduced adaptive responses following the high rate strain protocol cannot be clearly explained by the present experimental design, we can argue that this may be related to the time of the applied mechanical loading. It is well accepted that cyclic strain affects the homeostasis of tendinous tissue (Kjaer, 2004; Wang and Thampatty, 2006; Wang et al., 2012). Initiated by

Table 1. Comparison of the investigated parameters before (pre-exercise) and after (post-exercise) intervention 1, featuring the reference and high strain rate protocol and the respective effect sizes (*d*)

	Reference (*N*=14)			High strain rate (*N*=14)		
Parameter	Pre-exercise	Post-exercise	*d*	Pre-exercise	Post-exercise	*d*
CSA (mm²)	79.9±3.4	83.0±3.7*	0.23	80.5±3.9	82.5±3.6	0.14
Young's modulus (GPa)	0.91±0.07	1.43±0.17*	1.08	0.92±0.08	1.14±0.13	0.55
Length (mm)	49.3±5.4	49.7±3.8	0.02	52.5±5.8	52.9±5.5	0.02

The data are the mean ± s.e.m. of the average cross-sectional area (CSA) of the Achilles tendon (AT), Young's modulus of the AT and length of free AT (length).
*Statistically significant difference from the pre-exercise values (*P*<0.05).

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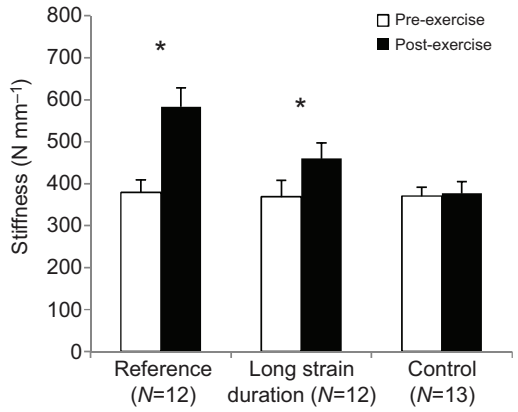

Fig. 3. Mean stiffness values and s.e.m. (error bars) of the AT before (pre-exercise) and after (post-exercise) intervention 2, featuring the reference and long strain duration protocol as well as the control group. There was a statistically significant interaction of intervention and protocol (P=0.002) indicating an increase following both exercise protocols and a pronounced increase following the reference compared with the long strain duration protocol (P=0.025). *Statistically significant difference between the pre- and post-exercise values (all P<0.008).

the applied stress, the external strain of the tendon is transmitted through the extracellular matrix on the cytoskeleton of the tendon cells, which trigger cellular and molecular responses (e.g. synthesis of collagen and matrix proteins), affecting the mechanical and morphological tendon properties (Galloway et al., 2013; Heinemeier and Kjaer, 2011; Wang, 2006). The viscoelastic properties of the extracellular matrix (Wang, 2006) may influence the time course of the external strain transmission to the tendon cells, indicating a time-dependent biological response. We suggest that the longer loading time during the reference protocol (3 s) may result in a more efficient transmission of the external tendon strain and, therefore, a higher magnitude of strain on the tendon cells compared with the shorter loading times during one-legged jumping (~0.26 s, high strain rate protocol). A possible greater transfer of the external tendon strain magnitude to the cellular level might be the reason for the superior adaptive responses of the tendon properties following the reference protocol. In agreement with the present findings, the results of our earlier studies (Arampatzis et al., 2010; Arampatzis et al., 2007) showed a pronounced adaptation of the AT properties following an exercise intervention using a low strain frequency with longer strain duration per contraction compared with a high strain frequency with shorter strain duration per contraction (i.e. 0.17 Hz, 3 s loading/3 s relaxation versus 0.5 Hz, 1 s loading/1 s relaxation). These findings indicate that increased hydrostatic pressure and fluid flow may not be as significant for tendon adaptation compared with the duration of the repetitive tendon loading. To our knowledge, the potential effect of strain rate, as an independent mechanical stimulus for tendon adaptation, has not been investigated so far in humans *in vivo*. In the present study, one-legged jumps were used to increase the strain rate of the AT compared with a reference protocol of the same loading magnitude and volume. Studies investigating the effects of plyometric training on AT properties found unchanged mechanical and/or morphological properties after an exercise intervention (Fouré et al., 2012; Fouré, 2011; Fouré, 2010; Houghton et al., 2013; Kubo et al., 2007) or less adaptive responses compared with an isometric protocol featuring longer loading duration (Burgess et al., 2007). The above-mentioned reports from the literature and the findings of the present study may indicate that plyometric training using jumps does not provide an optimal mechanical stimulus for tendon adaptation compared with training using longer durations of repetitive loading. However, the statistical tendency towards increased AT stiffness and average CSA as well as the effect size of 0.69 of the AT stiffness increase suggest that plyometric training may also induce adaptive responses. These adaptations might have become manifest, for instance, after a longer intervention duration as used in the present study (i.e. 14 weeks).

In the second intervention, we found clear adaptations of AT stiffness, which was a result of significant tendon hypertrophy and changes of the tendon material properties (i.e. increase in Young's modulus) in both protocols, demonstrating that the applied mechanical loading by means of the reference and the long strain duration protocol training effectively stimulated adaptive responses of the AT. Based on the viscoelastic properties of the extracellular matrix (Wang, 2006) and the time-dependent interaction between the extracellular matrix and the cytoskeleton, we proposed that a longer strain duration (12 s versus 3 s) may enhance the adaptation of mechanical and morphological tendon properties. However, the increase of AT stiffness and Young's modulus was more pronounced following the reference compared with the long strain duration protocol (AT stiffness: 54% versus 25% and d=1.51 versus 0.68; Young's modulus: 45% versus 18% and d=1.31 versus 0.57), indicating that the beneficial effect of the strain duration on tendon adaptation is limited and that repetitive loading in combination with an appropriate strain duration facilitates the adaptive response of

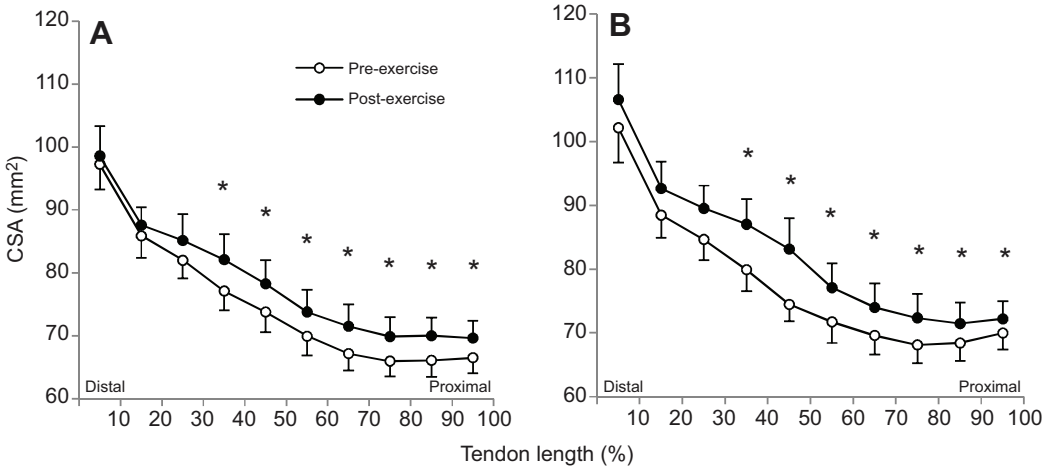

Fig. 4. Mean CSA and s.e.m. (error bars) of the AT in 10% intervals of the tendon length before (pre-exercise) and after (post-exercise) intervention 2, featuring the reference and long strain duration protocol. (A) Reference protocol (N=12); (B) long strain duration protocol (N=12). *Statistically significant difference between the pre- and post-exercise values (P<0.05).

Table 2. Comparison of the investigated parameters before (pre-exercise) and after (post-exercise) intervention 2, featuring the reference and long strain duration protocol and the respective effect sizes (*d*)

	Reference (*N*=12)			Long strain duration (*N*=12)		
Parameter	Pre-exercise	Post-exercise	*d*	Pre-exercise	Post-exercise	*d*
CSA (mm²)	75.4±2.6	78.8±3.0*	0.35	78.1±3.1	82.4±3.6*	0.38
Young's modulus (GPa)‡	0.97±0.08	1.41±0.11*	1.31	0.89±0.08	1.05±0.08*	0.57
Length (mm)	56.5±3.9	56.0±3.8	–0.03	54.2±3.7	53.8±3.8	–0.03

The data are the mean ± s.e.m. of the average cross-sectional area (CSA) of the Achilles tendon (AT), Young's modulus of the AT and length of free AT (length).
*Statistically significant difference from the pre-exercise values (P<0.05).
‡Statistically significant interaction (intervention × protocol) (P=0.021).

tendons. It seems that the external tendon strain was effectively transmitted on the tendon cells during the 3 s loading, 3 s relaxation protocol (i.e. reference protocol) and that longer strain duration did not provide a superior adaptive effect. Therefore, we can argue that a certain duration of strain is a crucial component of an effective stimulus for tendon adaptation and, under this premise, repetitive strain application can provide advantageous adaptive responses compared with a longer duration of static loading with fewer loading cycles. In accordance with this proposal, a recent study (Scott et al., 2011) reported a greater increase of tenocyte gene expression *in vitro* following cyclic (0.1 Hz) compared with static mechanical loading (5% strain) after 3 weeks in culture. This finding is in agreement with our *in vivo* experiments in which we applied a cyclic strain of 0.17 Hz (i.e. 3 s loading, 3 s relaxation) in the reference protocol, which resulted in a greater adaptive response in comparison to long static loading. In a similar manner, a beneficial effect of repetitive loading compared with static loading has already been reported regarding bone adaptation (Burr et al., 2002; Hert et al., 1971; Robling et al., 2001), giving further evidence for the superior effect of repetitive loading on the adaptive responses of biomaterials.

Besides a change in Young's modulus (i.e. material properties), our results also revealed a significant increase in the CSA of the AT following the reference and long strain duration protocol training. Several studies in the past years reported increases in tendon CSA following long-term exercise-induced loading, indicating that hypertrophy is an important mechanism for tendon adaptation (Arampatzis et al., 2007; Couppé et al., 2008; Houghton et al., 2013; Kongsgaard et al., 2007; Seynnes et al., 2009). Furthermore, the loading applied in the exercise protocols of the present study (i.e. 90% of maximal isometric force) was higher compared with that in other protocols (i.e. 70–80% of concentric one repetition maximum) reported in the literature (Kongsgaard et al., 2007; Seynnes et al., 2009). With regard to the findings that tendon hypertrophy is dependent on the load magnitude during training (Arampatzis et al., 2007), we can argue that the stimulus applied in our experiments is effective in facilitating tendon hypertrophy. Furthermore, we can exclude fluid accumulation after the last training session being responsible for the higher tendon CSA found in the post-exercise measurements because (a) the magnetic resonance images were recorded no less than 4 days after the final training bout, indicating an appropriate time for tissue recovery and (b) the increase of tendon CSA was accompanied by an increase in Young's modulus, giving evidence for altered material properties (e.g. higher collagen content) independent of the CSA.

There might be some methodological limitations in our study. First, in all three protocols (i.e. reference, high strain rate and long strain duration), we aimed to induce a high strain magnitude of the AT by means of a target force level of 90% of the maximal voluntary isometric plantar flexion force, with regard to the findings of our earlier experiments (Arampatzis et al., 2010; Arampatzis et al., 2007). However, the individual strain magnitude was not controlled during the interventions and, thus, may not have been exactly the same for every participant. The data from our pre- and post-exercise measurements show that the applied AT strain at 90% of the maximal voluntary isometric plantar flexion force was not significantly different between the experimental protocols (P>0.05; intervention 1: reference 6.63±1.24%, high strain rate 6.43±1.18%; intervention 2: reference 6.49±1.49%, long strain duration 6.94±1.54%; mean strain of pre- and post-exercise measurement ± s.d.). Therefore, we suggest that individual deviations in strain magnitude did not affect our conclusions. Furthermore, the interventions were conducted using a homogeneous sample (i.e. healthy young adults) to avoid the influence of cofactors (e.g. age, gender). Thus, it still needs to be shown how far our inferences extend to other populations, for example female adults or the elderly. Second, to investigate the effect of the four parameters of the mechanical stimulus (strain magnitude, strain frequency, strain rate and strain duration) on tendon adaptation by means of two parameter conditions (i.e. low and high), we conducted a total number of seven interventions instead of 16 (4 parameters ^ 2 conditions). Because our first experiments (Arampatzis et al., 2010 Arampatzis et al., 2007) showed that only the high strain magnitude protocols induced adaptive responses of the tendon, we decided to apply only the high strain magnitude condition in our present experimental design. Hence, the most effective training protocol we could identify in our first experiments (i.e. high strain magnitude and low frequency) was compared with a modulation of the strain rate and strain duration to show a superimposed effect of these two parameters. By means of this systematic research approach, we were able to significantly reduce the number of necessary training protocols (from 16 to seven) without a decrease in scientific quality.

The present results, in combination with our previous experiments (Arampatzis et al., 2010 Arampatzis et al., 2007), demonstrate that a high strain magnitude beyond habitual loading must be applied to the tendon to induce adaptive responses of the mechanical, morphological and material tendon properties. A tendon strain duration of about 3 s seems to be necessary for effective transmission of the external tendon strain at the cellular level and, therefore, plyometric exercises like jumping may not be an optimal training stimulus for tendon adaptation. Furthermore, the advantageous effect of longer tendon strain duration (i.e. >3 s) seems to be limited and repetitive application of strain provided a more effective stimulus for tendon adaptation in young healthy male adults.

MATERIALS AND METHODS

Subjects

Thirty-nine male adults participated in the present study after giving informed consent to the experimental procedure, which was approved by the

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local ethics committee. The participants were randomly assigned to one of the two experimental groups (group 1: *N*=14, age 26.7±4.2 years, mass 82.2±13.1 kg, height 182.3±5.3 cm; group 2: *N*=12, age 29.5±3 years, mass 74.8±7.3 kg, height 177.6±7 cm) or to a control group that received no specific training (*N*=13, age: 26.5±4.5 years, mass: 78.6±10.7 kg, height: 182.3±10.7 cm). All participants reported no musculoskeletal impairments of the lower limbs and were physically active but not involved in high-performance sports.

A statistical power analysis was performed *a priori* using the AT stiffness and Young's modulus values (*N*=11) from the most effective exercise protocol of our previous intervention experiments (Arampatzis et al., 2010; Arampatzis et al., 2007) to calculate the required sample size for an appropriate statistical power. The analysis (α=0.05, power=0.95, correlation=0, effect size: stiffness 1.6, Young's modulus 1.2) by means of the software G*Power (version 3.1.9.2, Germany) revealed that a sample size of *N*=12 would be sufficient to achieve a high statistical power (i.e. 0.95) of the expected outcome.

Exercise interventions

To investigate the effect of the strain rate and strain duration on tendon adaptation, two separate exercise interventions were conducted for 14 weeks with four sessions per week. Following a standardized warm-up, five sets of plantar flexion contractions were used to induce cyclic strains of the AT. In both interventions, one randomly assigned leg of each participant was exercised following a reference protocol similar to the one that induced the most superior adaptive effects of the AT mechanical and morphological properties in our earlier studies (Arampatzis et al., 2010; Arampatzis et al., 2007). The reference protocol included repetitive isometric contractions (4× 3 s loading, 3 s relaxation; Fig. 5) on a leg press (ankle angle 5 deg dorsal flexion, knee joint fully extended and hip flexed at 115 deg). In the first intervention (group 1), the contralateral leg was trained by means of one-legged jumps (72 per set), thus increasing the strain rate with respect to the reference protocol (high strain rate protocol, Fig. 5). The short contact phases during jumping were associated with an approximately three times greater rate of force development compared with the isometric contractions of the reference protocol (time to peak force was ~130 ms and during the reference isometric contractions it was ~380 ms), indicating a higher strain rate of the AT compared with the reference protocol. With regard to our earlier findings (Arampatzis et al., 2010; Arampatzis et al., 2007), we set the target force in both the reference and high strain rate protocol to 90% of the maximal voluntary isometric plantar flexion force (MVC) to induce a high strain magnitude on the AT, i.e. 6.63±1.24% and 6.43±1.18% (mean of pre- and post-exercise measurements ± s.d.), respectively. The MVC target values were updated every 10 training sessions. A pilot study was conducted to compare the ankle joint moments during the one-legged jumps with the isometric condition and the results indicated similar joint moments and, thus, magnitude of tendon loading during the two conditions. The loading volume (integral of plantar flexion force over time) was kept similar in the two protocols by adjusting the number of contractions (i.e. 4× 3 s loading and 3 s relaxation in the reference protocol and 72 jumps in the high strain rate protocol) to ensure direct comparability (Fig. 5). Visual feedback of the exerted and target plantar flexion force was given to the participants during training (Fig. 5). Before starting both interventions, 2 weeks of reduced low intensity exercise were performed to accustom the participants to the specific load.

The second intervention (group 2) aimed to investigate the effect of a modification of strain duration on AT adaptation. The participants exercised one leg following the same reference protocol as applied during intervention 1 (repetitive isometric contractions, 4× 3 s loading, 3 s relaxation per set). In contrast, the other leg performed a single 12 s isometric plantar flexion contraction per set and, therefore, the strain duration applied on the AT was four times longer compared with the reference protocol (Fig. 5). Both the reference and long stain duration protocol included the high strain magnitude (i.e. 6.49±1.49% and 6.94±1.54%, respectively; mean of pre- and post-exercise measurements ± s.d.) and loading volume of the first intervention (i.e. 90% of the MVC). By means of this experimental design that featured the same magnitude and volume of loading in both interventions (i.e. strain rate and strain duration), we were able to compare directly the effects of strain rate and strain duration modulation on AT adaptation. Before and after the interventions, the mechanical, morphological and material properties of the AT of both legs were assessed. For the control group, the AT mechanical properties were measured at the left and right leg and both values were included in the further analysis.

AT morphological properties

The morphological properties of the free AT (i.e. length and CSA) were determined by means of magnetic resonance imaging (MRI). Only the participants of the two experimental groups (intervention 1 and 2) were investigated, as changes of the AT morphological properties of the control group participants without a specific stimulus were not expected (Kubo et al., 2010; Kubo et al., 2006). A 0.25 T magnetic resonance scanner (G-Scan, Esaote, Italy) captured transversal and sagittal magnetic resonance scans of the AT [3D HYCE (GR) sequence, TR 10 ms, TE 5 ms, flip angle 80 deg, slice thickness 3 mm, one excitation] while the participants lay in supine position with the hip and knee extended and the ankle fixed in a relaxed position. The sagittal images were used to detect the borders of the free AT, i.e. m. soleus–AT junction and initial attachment on the calcaneus bone, respectively (Fig. 6). Every transversal slice within these borders was segmented manually using the software OsiriX (Pixmeo SARL, version 2.5.1, Switzerland) in order to determine the CSA of the tendon (Fig. 6). Three independent observers analyzed the magnetic resonance images of both legs from all participants and the mean values from all observers were used for the further analysis. The free AT length was calculated as the

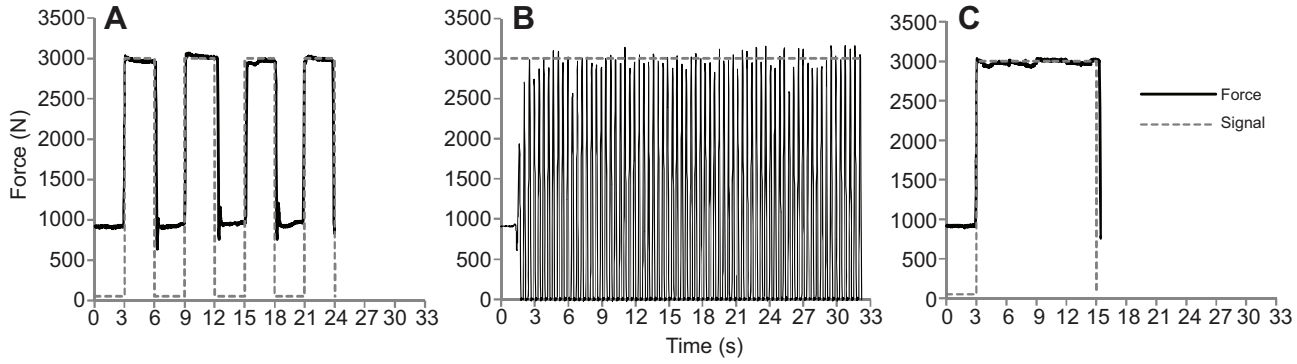

Fig. 5. Loading profiles of the reference protocol, the high strain rate protocol and the long strain duration protocol of the two interventions. Five sets were performed, 4 days per week for 14 weeks, featuring similar exercise volume (integral of the plantar flexion force over time). (A) The reference protocol: four repetitions of 3 s loading, 3 s relaxation; (B) the high strain rate protocol: 72 one-legged jumps; and (C) the long strain duration protocol: one repetition of 12 s loading. The rate was increased by a factor of ~3 and duration by a factor of 4 with respect to the reference protocol to investigate their effect on AT adaptation. Plantar flexion contractions at 90% maximal voluntary isometric plantar flexion force were used to induce high magnitude strain of the AT. Signal, signal displayed to the participants to control the magnitude and volume of loading (i.e. time and individual target force); force, plantar flexion force over time exerted during exemplary exercise for one participant.

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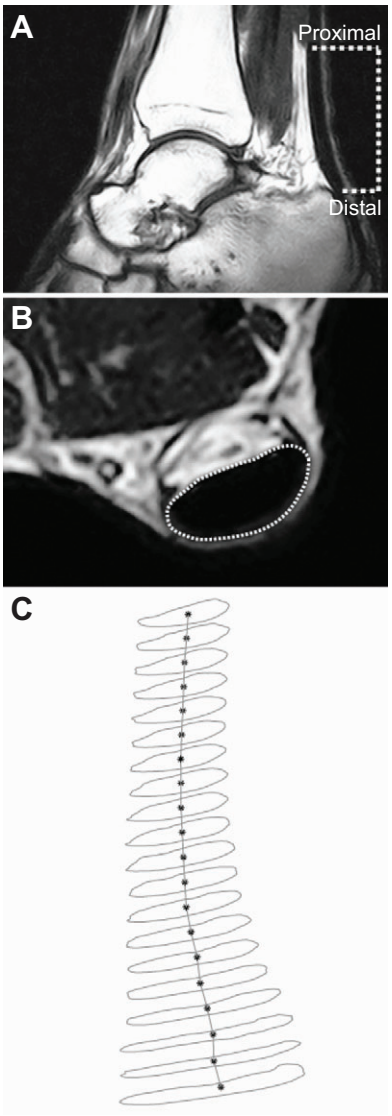

Fig. 6. Magnetic resonance images of the free AT. Sagittal (A) and transverse (B) images were used to investigate morphological AT properties (i.e. length and CSA; C). Transversal images served to detect the proximal (m. soleus–AT junction) and distal (initial attachment on the calcaneus bone) border of the free AT (A). Sagittal images within this range were segmented to determine the CSA (B) and to calculate the tendon length (i.e. the curved path through the CSA centroids; C).

curved path through the centroids of the CSAs, which were calculated using a Delaunay triangulation (Fig. 6). The CSA was displayed for 10% intervals along the free AT length to consider region-specific changes of the tendon (Arampatzis et al., 2007). The average CSA of the AT was calculated as the mean from all determined CSAs along the AT length.

AT mechanical and material properties

The stiffness and Young's modulus of the AT were examined through a combination of dynamometry, MRI and ultrasound measurements. The AT force was calculated from the ankle joint moment and the tendon lever arm. The participants performed maximal isometric plantar flexion contractions in a seated position with the knee extended and the ankle angle at a neutral position (tibia perpendicular to the sole of the foot, 90 deg) on a dynamometer (Biodex-System 3, Biodex Medical Systems Inc., USA). To account for axis misalignments of the ankle joint and dynamometer during the MVCs, the resultant joint moments were calculated by means of inverse dynamics (Arampatzis et al., 2005). The relevant kinematic data were captured by an infrared motion capture system (Vicon Nexus, version 1.7.1, Vicon Motion Systems, UK) integrating nine cameras operating at 250 Hz. Furthermore, the contribution of the antagonistic muscle tibialis anterior to the measured ankle joint moments was taken into account (Mademli et al., 2004). The activity of the tibialis anterior muscle during the maximum plantar flexions was recorded by means of electromyography (EMG; Myon m320RX, Myon AG, Switzerland). Based on the relationship of EMG amplitude of the m. tibialis anterior and the exerted moments during sub-maximal isometric dorsal flexion contractions, the corresponding antagonistic moment during the maximal plantar flexion could be calculated (Mademli et al., 2004). The AT force was calculated by dividing the ankle joint moment by the AT lever arm, which was determined by applying the tendon excursion method (An et al., 1984). This method is based on the ratio of the m. gastrocnemius medialis myo-tendinous junction (MTJ) displacement obtained by B-mode ultrasonography to the corresponding angular excursion of the ankle joint (Fath et al., 2010). Although this method does not account for the tendon compliance, the magnitude of tendon elongation due to the ankle angle change was reported to be low in the range used for the lever arm calculation (i.e. 5 deg dorsal flexion to 10 deg plantar flexion) (De Monte et al., 2006). Alterations of the tendon lever arm during the contraction were considered in the calculation using the factor suggested by Maganaris et al. (Maganaris et al., 1998). The elongation of the AT during a ramped MVC (~5 s gradual increase of force) was measured by capturing the MTJ displacement using B-mode ultrasonography. A 10 cm linear probe (My Lab 60, Esaote, Italy) embedded in a custom-built foam cast was fixed to the shank and recorded the displacement of the MTJ at 25 Hz. Afterwards, the displacement was traced manually frame by frame within a custom-written MATLAB interface (The MathWorks, version 2012, USA). Displacements of the MTJ as a result of changes in the ankle joint angle during the MVC were subtracted, as they significantly affect the tendon elongation measurement (Arampatzis et al., 2008). For this purpose, the passive MTJ displacement in relation to the ankle angle was analyzed in an additional trial (inactive ankle rotated over the full range of motion of the ankle joint at 5 deg s^{-1}). With regard to the reliability of ultrasound-based tendon elongation measurements reported by Schulze et al. (Schulze et al., 2012), we averaged the force and elongation data of five contractions.

The AT stiffness was calculated from the tendon force and tendon elongation ratio between 50% and 100% of the maximum tendon force using linear regression. The tendon rest length was measured from the tuberositas calcanei to the MTJ at an ankle angle of 110 deg (plantar flexed) and extended knee, as in this position slackness of the inactive gastrocnemius medialis muscle–tendon unit has been reported (De Monte et al., 2006). The Young's modulus of the AT was calculated from the relationship of tendon stress and tendon strain from 50% to 100% of the maximum stress by means of linear regression. The AT stress was calculated as the quotient from the AT force and the averaged CSA and the AT strain as the quotient from the elongation and the rest length.

Statistics

Normal distribution of the data was examined using the Kolmogorov–Smirnov test. An analysis of variance for repeated measures (RM-ANOVA) was performed separately for both interventions in order to determine the effect of the intervention (within-subjects variable: before and after) as well as the effect of the different protocols (between-subjects factor: reference, high strain rate or long strain duration and control) on the AT stiffness. A Bonferroni *post hoc* analysis was conducted in the case of a significant interaction of the factors intervention and protocol. A similar RM-ANOVA was applied on the average CSA and Young's modulus values; however, the values of the control group were not present and, therefore, this group was not included in the between-subjects factor. The 10% intervals of the CSA along the AT length were tested for pre- and post-intervention differences using a paired *t*-test. The statistics were performed using the software SPSS Statistics (IBM, version 20, USA) and the level of significance for all statistical procedures was set at α=0.05. Furthermore, to estimate the strength of potential alterations of the investigated parameters following the interventions, the effect size (*d*) was calculated. Values of <0.20 indicate small effect sizes, those of 0.50 indicate medium effect sizes, and values >0.80 indicate large effect sizes (Cohen, 1988).

Competing interests
The authors declare no competing financial interests.

Author contributions
S.B. conceived, designed and executed the experiments, interpreted the findings, and drafted and revised the article; F.M. executed the experiments, interpreted the findings, and drafted and revised the article; M.T. executed parts of the experiments; M.K. drafted and revised the article; A.A. conceived and designed the experiments, interpreted the findings, and drafted and revised the article.

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